NASA CR-112020

MDC G2828

17-KETOGENIC STEROIDS EXCRETION IN CREWMEN IN A 90-DAY MANNED TEST OF AN ADVANCED REGENERATIVE LIFE SUPPORT SYSTEM

FEBRUARY 1972

Prepared Under Contract No. NAS1-10717

By
Biotechnology and Power Department
McDonnell Douglas Astronautics Company
Huntington Beach, California

For
LANGLEY RESEARCH CENTER
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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SUMMARY

This study was carried out to assess a possible correlation between stress and urinary levels of 17-Ketogenic Steroids (17-KGS) and the Sodium/Potassium ratio (Na/K). 17-KGS and Na/K were determined in 19 urine specimens collected by each of 4 crewmen during the 90-day test. The specimens represented 10% aliquots of 24-hour collections stored frozen onboard the simulator untill passthrough. Electrolytes were analyzed immediately after sample passthrough while the steroids were determined post-test on aliquots of the original sample held at 203°K. Steroid data was corrected for body weight and also for analytical variation in the laboratory urine pool control.

Long-term nonspecific responses to low-level stress appear to be reflected by the individual and group mean 17-KGS excretion patterns. The first 39 and the last 20 days of the test were significantly different ($P<.05$)--and presumably more stressful to the crew--than the period from days 39 to 67. Reduction of adrenocortical function during the mid-test phase is attributed to either an adaptation to chronic or intermittent stress or was the result of an actual reduction in the operational demands of the test during this time. Although the all-crew mean Na/K and 17-KGS excretion curves appear to parallel each other during the mid-test period, the correlation coefficient between these parameters was only 0.54. Most remarkable of the metabolic findings is the prevalance of high Na/K ratios (mean ratio at each sample was never below 3.0) and an abrupt peak on day 74 for all 4 crewmen. Lack of a high correlation between Na/K and 17-KGS is probably due to several factors, prime of which appears to be related either to a time lag in the differential response to the same stress and/or a greatly individual response by the Na/K ratio as compared with a group tendency for the 17-KGS.

INTRODUCTION

This study was prompted by an observation during the last third of the 90-day test that an abrupt peak in urinary Na/K coincided with the apparent resolution of a prolonged period of morale decline (Reference 1).

The availability of urine specimens throughout the test provided an opportunity to evaluate and possibly confirm psychological, sociological, and metabolic indications of stress levels on or about test day 74 and at other times by assessing adrenocortical function.

Evidence of endocrine-metabolic hyperactivity, which suggests non-specific stress, has been described in various circumstances involving flying personnel (References 2 and 3) and air traffic controllers (Reference 3). Daily, weekly, and seasonal variations in endocrine-metabolic activity have also been established under essentially non-stressful conditions (References 4, 5, and 6). Although the latter "baseline" type of data was not available in this study, the test phase results alone were considered sufficient to relate significant trends in metabolic and endocrine parameters with psychological determinants of the relative level of stress.

SECTION I

MATERIALS AND METHOD

Urine specimens were collected by the crewmen in plastic bags as 10% aliquots of each void over a 24-hour period. Samples were collected once per week for the first 8 weeks of the test and twice per week thereafter. Urine volume was measured after voiding into a volumetric cylinder and recorded on the sample bag. The sample bags were stored in an onboard freezer at 269°K throughout the collection period and transferred to the outside laboratory on a once-per-week passthrough schedule. Electrolytes were determined by flame photometry shortly after passthrough by a contract laboratory. Residual urine specimens were refrozen and held at 203°K until post-test analysis of 17-KGS was initiated. Batches of samples numbering 4-12 samples per batch were analyzed in chronological order starting at the beginning of the test. Dehydroepiandrosterone standards and a duplicate urine pool control were carried through the analytical process with each batch of samples. The 17-KGS analytical method is described in Reference 7, and modifications in References 8, 9, and 10.

SECTION II

RESULTS

Table 1 contains the results of the sodium and potassium analyses expressed as the ratio Na/K (mEq/mEz). Also included are the means and standard deviations ($\bar{x}\pm s$) for the crew of four at each sampling period and for the entire test, as well as individual and group crew values for the entire test. Table 2 presents in a similar fashion the 17-KGS data.

Analysis of mean Na/K and 17-KGS values for significant differences between crewmen and groups of crewmen is presented in Tables 3 and 4. This analysis indicates that CM 2 was significantly different from CM 3 with respect to both Na/K ($P<.02$) and 17-KGS ($P<.10$) mean excretion rate. Mean values for the other crewmen were not signficantly differentiated. Comparing the day with the night crew, it is noted that there is no significant difference in 17-KGS excretion at any test phase or for the test as a whole but there is day vs night crew differentiation for Na/K for the test as a whole ($P<.05$). Examination of the data for intra-crew significant differences (according to sample number), reveals that the day crew Na/K was significantly higher during test days 74-90 than during test days 46-70; the night crew showed no differences in Na/K between test thirds. The 17-KGS data reveals that the middle of the test was significantly differentiated from the initial and terminal phases for both crews.

TABLE 1

Na/K

Test Day (Spl.#)	CM 1	CM 2	CM 3	CM 4	All Crew $\bar{x}$ ±(s) by Sample #
4	4.83	4.31	4.52	3.64	4.32 (0.50)
11	3.73	3.24	2.70	3.03	3.17 (0.43)
18	3.37	3.32	2.62	3.96	3.32 (0.55)
25	4.65	3.99	2.52	4.02	3.79 (0.90)
32	4.11	4.74	3.53	4.76	4.28 (0.59)
39	3.11	4.16	3.35	3.04	3.41 (0.51)
46	3.14	4.37	3.20	3.12	3.46 (0.61)
53	2.74	5.00	3.49	4.20	3.86 (0.97)
56	2.88	5.00	2.41	3.69	3.49 (1.13)
60	3.47	4.06	3.98	4.61	4.03 (0.47)
63	4.00	4.06	4.09	4.41	4.14 (0.18)
67	2.83	3.29	2.97	3.14	3.06 (0.20)
70	3.57	3.95	3.30	2.58	3.35 (0.58)
74	6.24	6.36	5.07	5.33	5.75 (0.64)
77	3.74	3.64	3.90	4.35	3.91 (0.31)
81	3.62	3.32	2.52	3.04	3.12 (0.47)
84	3.83	4.74	5.35	3.62	4.38 (0.81)
88	4.96	4.37	4.24	2.88	4.11 (0.88)
90	4.35	5.14	3.72	4.15	4.34 (0.59)
Test $\bar{x}$	3.85	4.27	3.55	3.77	
(± s)	(0.87)	(0.79)	(0.85)	(0.74)	

	CM 1 & 2	CM 3 & 4	All Crew
Test $\bar{x}$	4.06	3.66	3.86
(± s)	(0.85)	(0.80)	(0.84)

TABLE 2

CORRECTED 17-KGS DATA(μg/Kg/24 HRS)

Test Day (Spl. #)	CM 1	CM 2	CM 3	CM 4	All Crew $\bar{x} \pm (s)$ by Sample #
4	122.8	170.6	179.1	163.1	158.9 (24.9)
11	173.9	189.2	192.9	212.5	189.6 (11.9)
18	172.2	205.6	165.1	176.2	179.8 (17.8)
25	197.9	209.2	171.4	205.1	195.9 (17.0)
32	160.7	189.9	185.6	193.2	182.3 (14.8)
39	181.4	157.4	252.0	192.9	195.9 (40.2)
46	147.5	136.3	129.8	130.1	135.9 (8.3)
53	138.7	109.5	164.3	162.6	143.8 (25.7)
56	112.9	112.4	129.0	160.8	128.8 (22.7)
60	233.5	137.3	140.2	149.3	165.1 (45.9)
63	147.1	125.1	199.9	160.1	158.0 (31.4)
67	144.8	151.4	163.1	149.8	152.3 (7.7)
70	212.3	155.5	133.3	170.4	167.9 (33.3)
74	258.9	179.0	206.0	163.7	201.9 (41.8)
77	257.9	163.5	218.5	176.2	204.0 (42.9)
81	191.9	167.2	243.7	210.0	203.2 (32.2)
84	210.7	201.8	215.9	169.4	199.4 (20.9)
88	191.9	201.1	243.7	211.8	212.1 (22.6)
90	138.6	97.3	150.2	152.3	134.6 (25.6)
Test $\bar{x}$ (± s)	178.7 (42.7)	161.0 (34.4)	183.3 (39.2)	173.6 (23.1)	

	CM 1 & 2	CM 3 & 4	All Crew
Test $\bar{x}$ (± s)	169.9 (39.3)	178.5 (32.1)	171.1 (34.5)

TABLE 3

"t" TEST, MEAN Na/K

Crewman, All Samples	"t"	P	(N)
1 vs 2	-1.56	NS	(19)
1 vs 3	1.07	NS	(19)
1 vs 4	0.30	NS	(19)
2 vs 3	2.70	<.02	(19)
2 vs 4	2.01	NS	(19)
3 vs 4	-0.84	NS	(19)
Day vs Night Crew			
All Samples	2.12	<.05	(38)
TD 4-39	1.78	NS	(12)
TD 46-70	0.86	NS	(14)
TD 74-90	1.29	NS	(12)
Day Crew			
TD 4-39 vs 46-70	0.82	NS	(13)
TD 4-39 vs 74-90	-1.65	NS	(12)
TD 46-70 vs 74-90	-2.27	<.05	(13)
Night Crew			
TD 4-39 vs 46-70	-0.14	NS	(13)
TD 4-39 vs 74-90	-1.58	NS	(12)
TD 46-70 vs 74-90	-1.59	NS	(13)

TABLE 4

"t" TEST, MEAN 17-KGS EXCRETION (µg/Kg/24 Hrs)

Crewman, All Samples	"t"	P	(N)
1 vs 2	1.41	NS	(19)
1 vs 3	-0.34	NS	(19)
1 vs 4	0.46	NS	(19)
2 vs 3	-1.86	<.10	(19)
2 vs 4	-1.32	NS	(19)
3 vs 4	0.93	NS	(19)
Day vs Night Crew			
All Samples	-1.04	NS	(38)
TD 4-39	-1.26	NS	(12)
TD 46-70	-0.51	NS	(14)
TD 74-90	-0.52	NS	(12)
Day Crew			
TD 4-39 vs 46-70	2.50	<.05	(13)
TD 4-39 vs 74-90	-0.72	NS	(12)
TD 46-70 vs 74-90	-2.58	<.05	(13)
Night Crew			
TD 4-39 vs 46-70	4.30	<.01	(13)
TD 4-39 vs 74-90	-0.58	NS	(12)
TD 46-70 vs 74-90	-4.15	<.01	(13)

The 17-KGS data collected in this study are presented graphically in Figures 1-4 and the Na/K data in Figures 7-10. Figure 12 compares the steroid and electrolyte ratio patterns by plotting the all-crew means for each sample over a common line representing the all-test mean for both parameters. As previously indicated in the statistical results, the period from test day 39-70 is marked by a pronounced fall-off in steroid excretion as compared to the average levels attained prior to and following this phase of the test. This is seen most clearly in Figure 4, a plot of the all-crew mean 17-KGS excretion pattern. Discrete phasing in mean 17-KGS excretion may be artifactual since the individual and group crew curves do not display such clear-cut differences between test thirds. It appears, however, that even the individual and group crew curves display an overall pattern for the test that may be characterized by an initial phase of increased steroid excretion, followed by an abrupt fall-off, and then a gradual return to high levels a few weeks prior to test termination.

The Na/K plot in Figure 12 is most markedly different from the 17-KGS pattern by virtue of the relative lack of "plateaus" at each end of the curve. Unlike the all-crew 17-KGS pattern, plotting the Na/K ratios for each 24-hour sample as an all-crew average did not result in discrete phasing. Peaks in the mean Na/K ratio are evident on or about test days 32, 63, 74, and 84, the most prominent and abrupt peak occurring on day 74. This data point is characteristically obvious for both groups of crewmen (Figure 9) in its magnitude as well as in its appearance following and preceding a marked depression. Although the steroid and electrolyte ratio curves appear to parallel each other between test day 46 and 74, the correlation coefficient for this 4-week period is only 0.54 as opposed to 0.17 for the entire test. Plots of the difference between all-crew actual and ideal accumulative excretion rates for 17-KGS and for Na/K

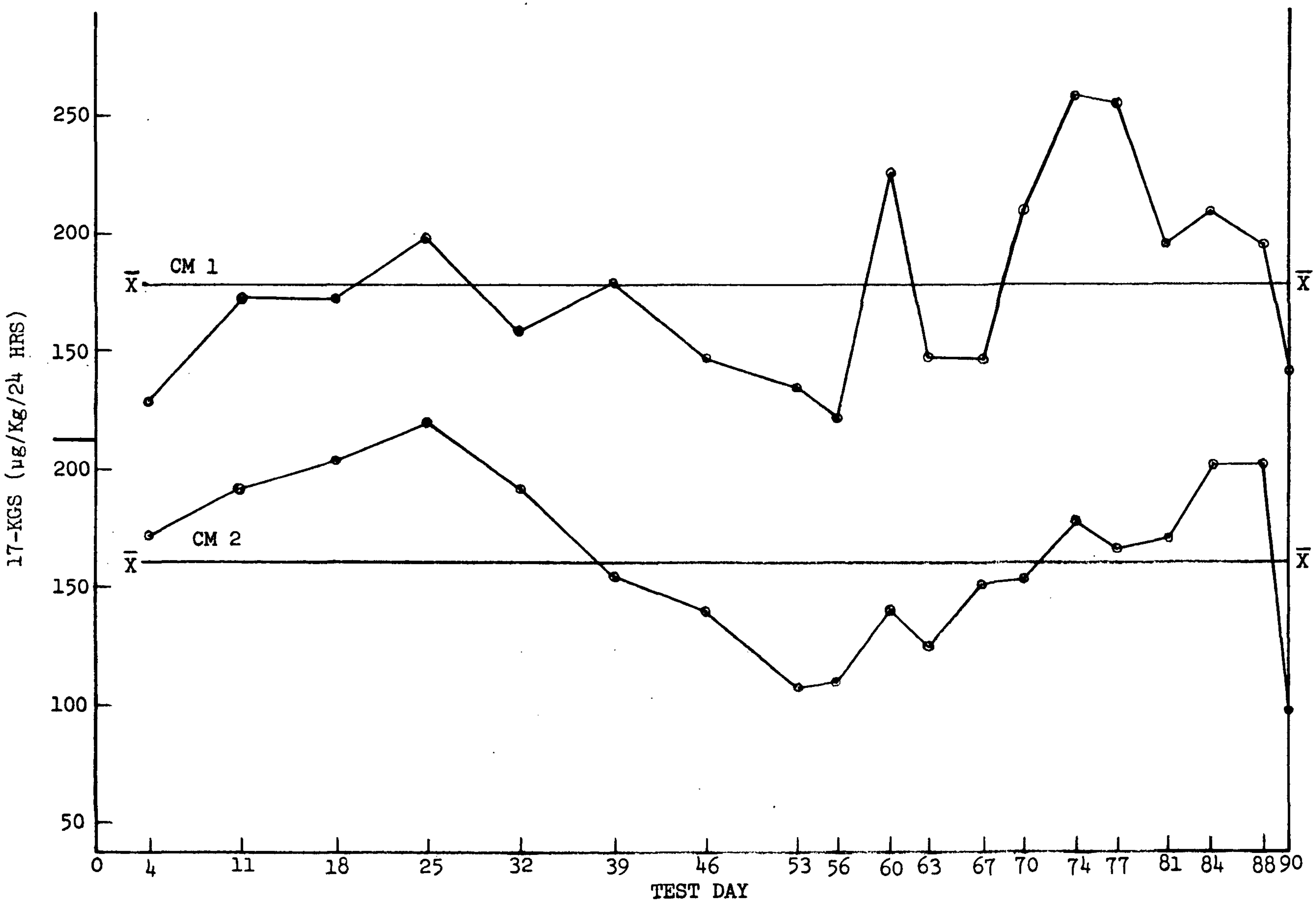

FIGURE 1. 17-KGS EXCRETION PER 24 HRS, CREWMEN 1 AND 2

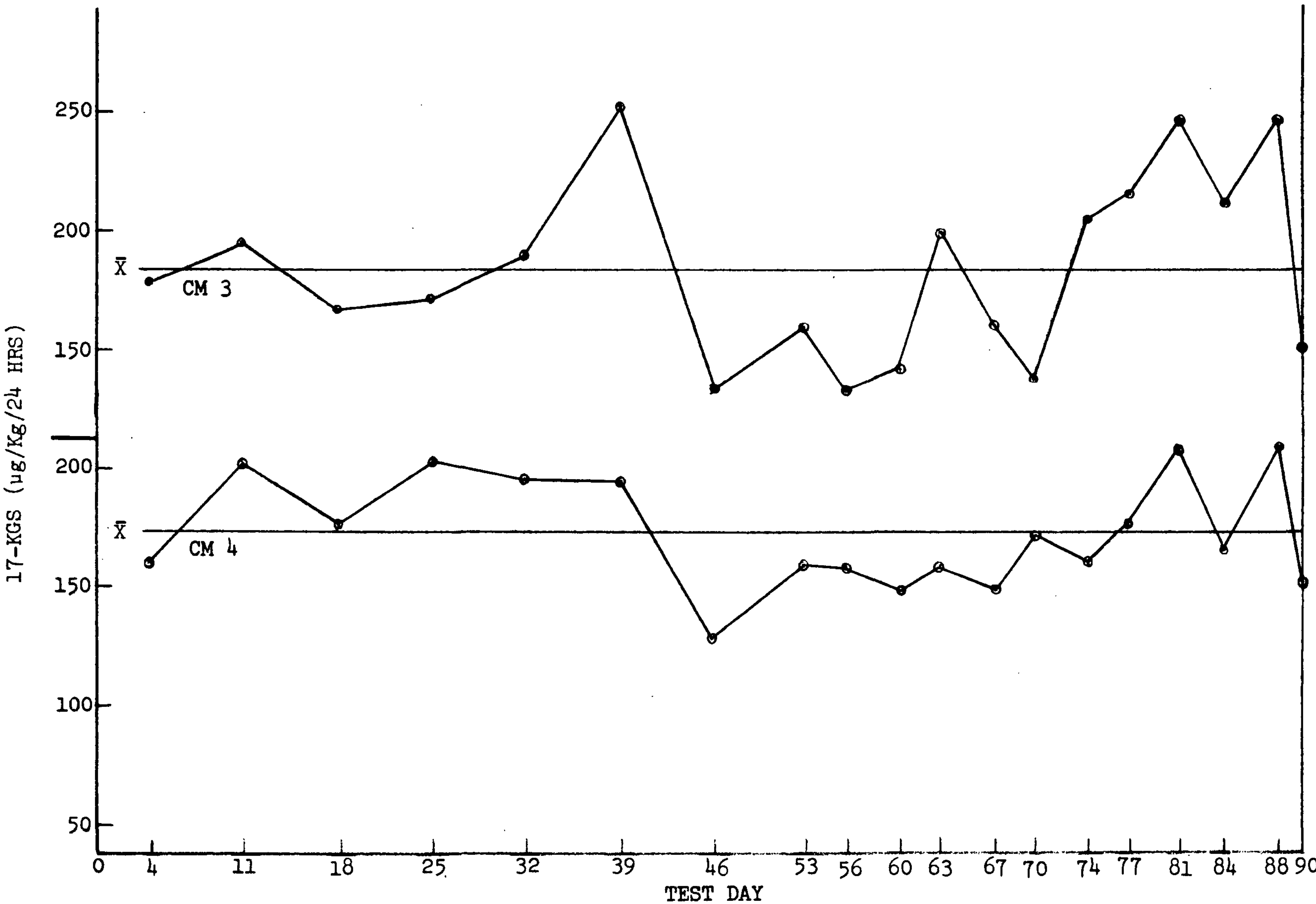

FIGURE 2. 17-KGS EXCRETION PER 24 HRS, CREWMEN 3 AND 4

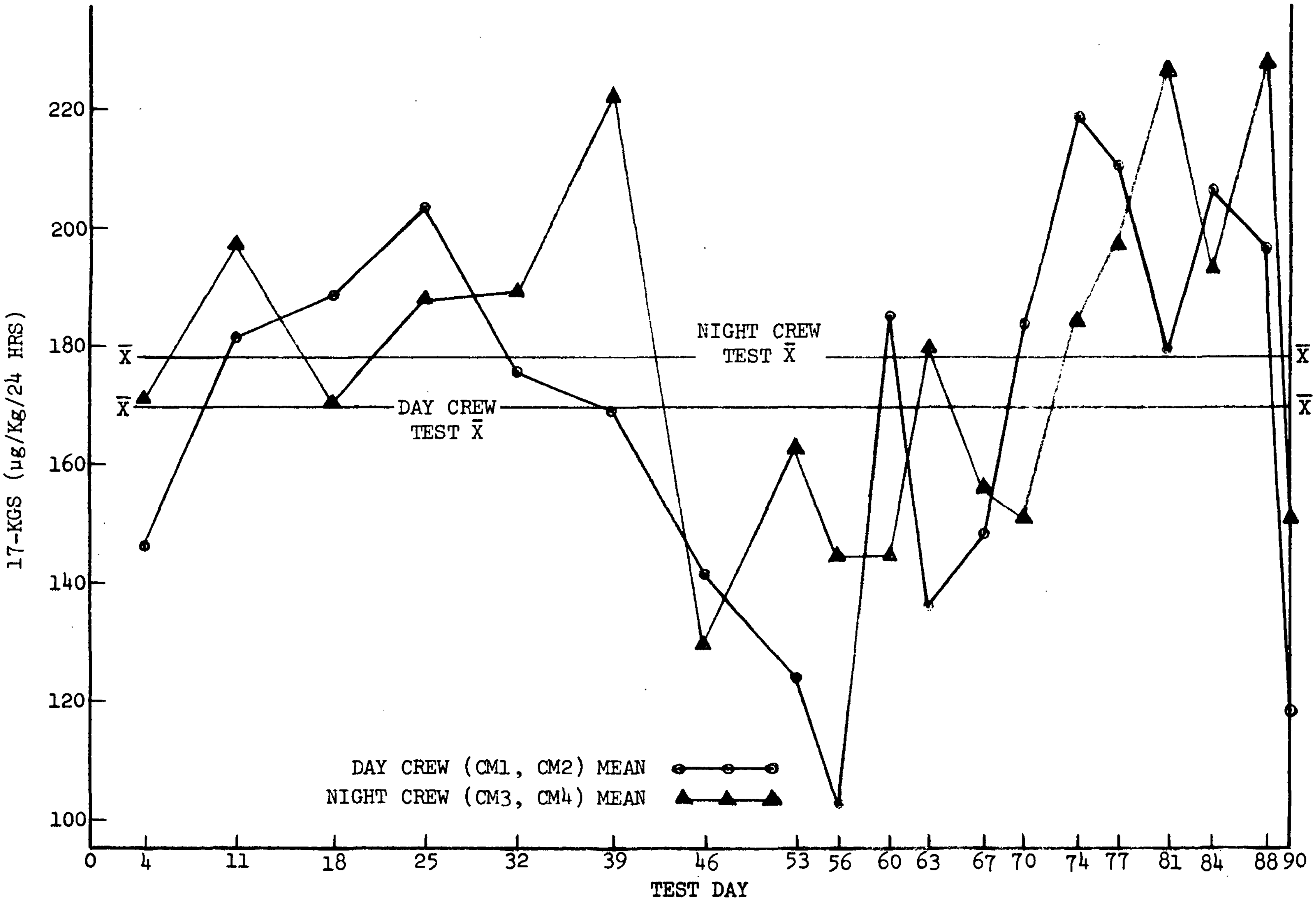

FIGURE 3. DAY VS. NIGHT CREW MEAN 17-KGS EXCRETION

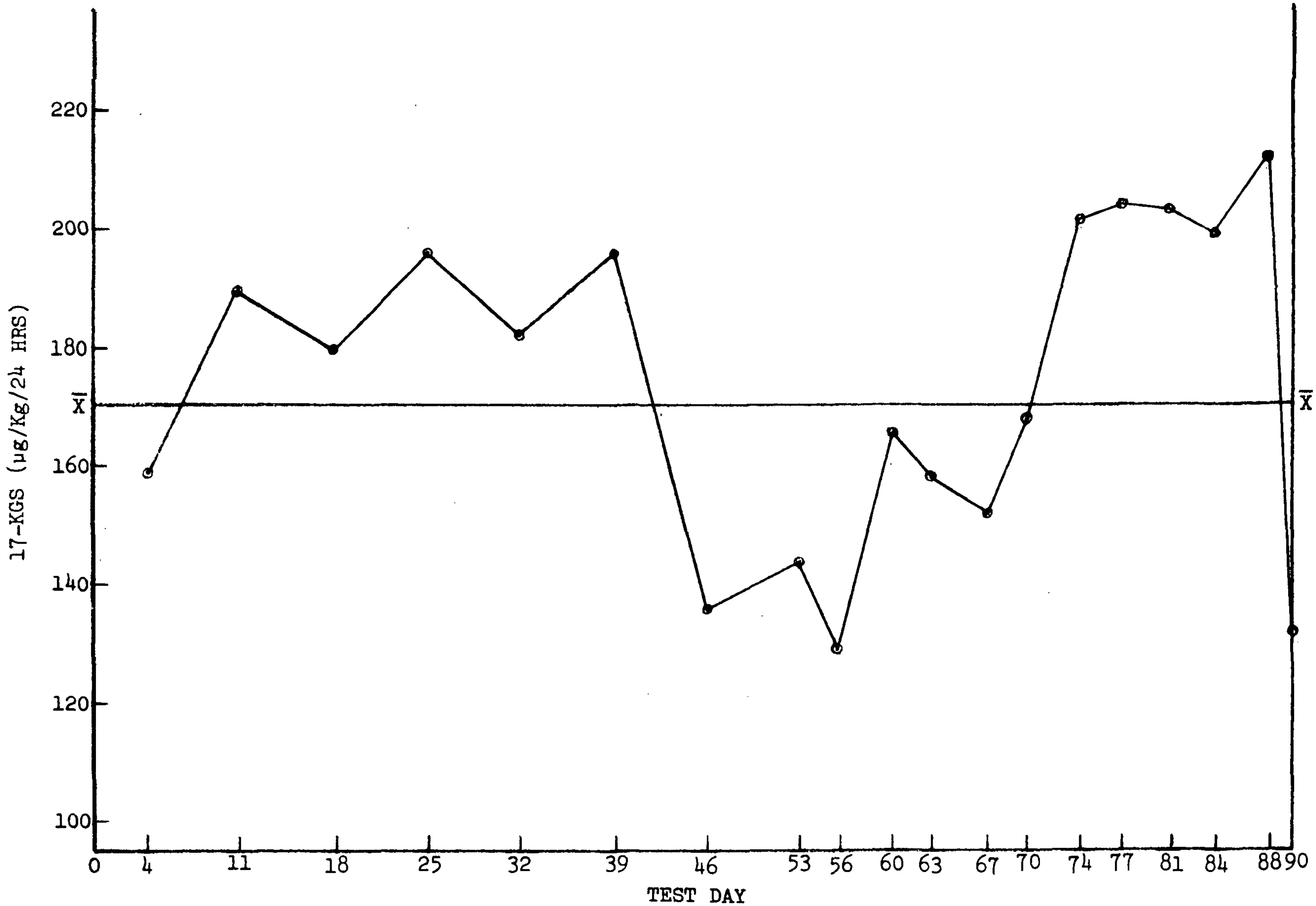

FIGURE 4. ALL CREW MEAN 17-KGS EXCRETION

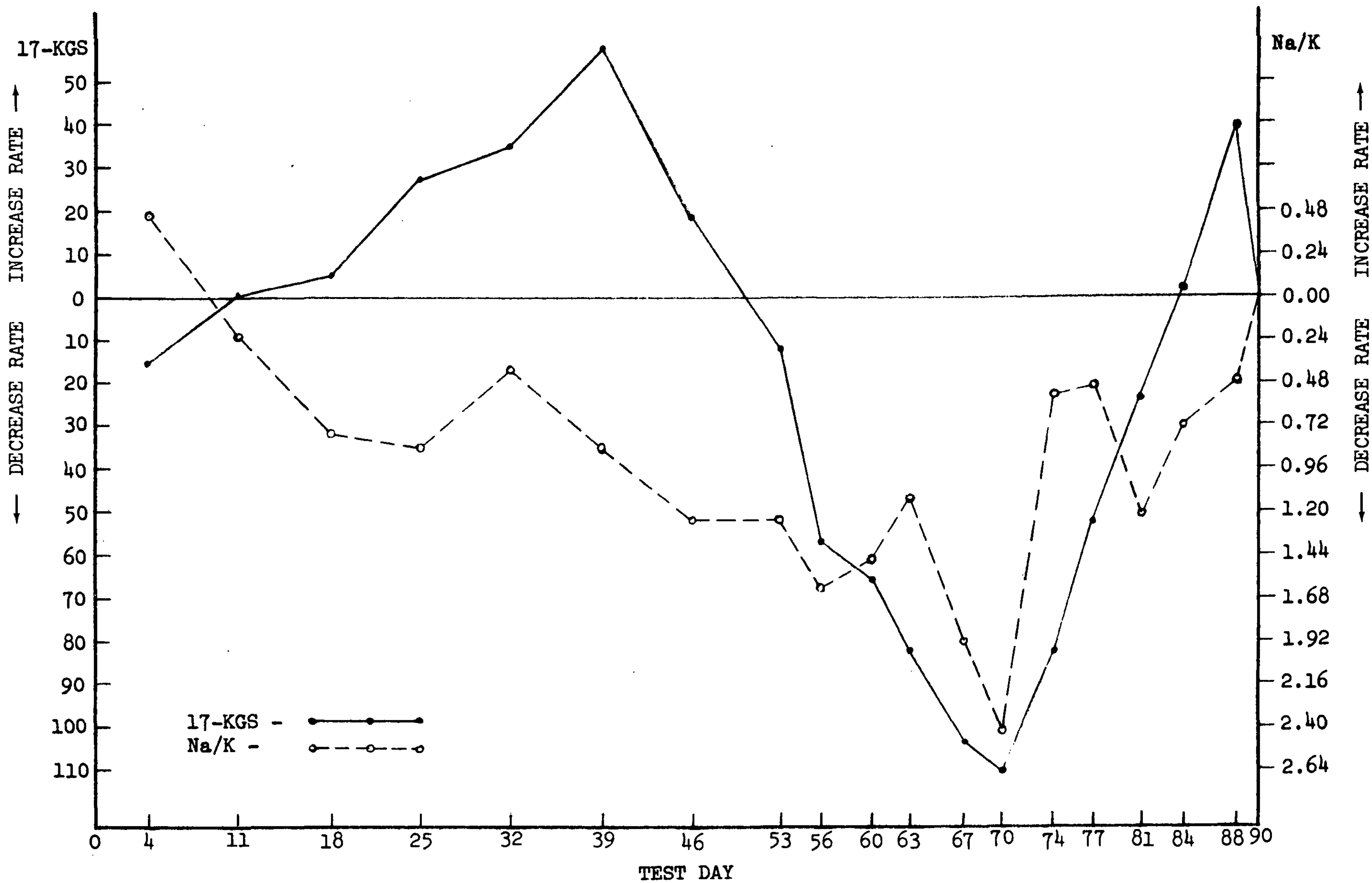

FIGURE 5. PLOT OF DIFFERENCE BETWEEN ALL CREW ACTUAL AND IDEAL ACCUMULATIVE EXCRETION RATE

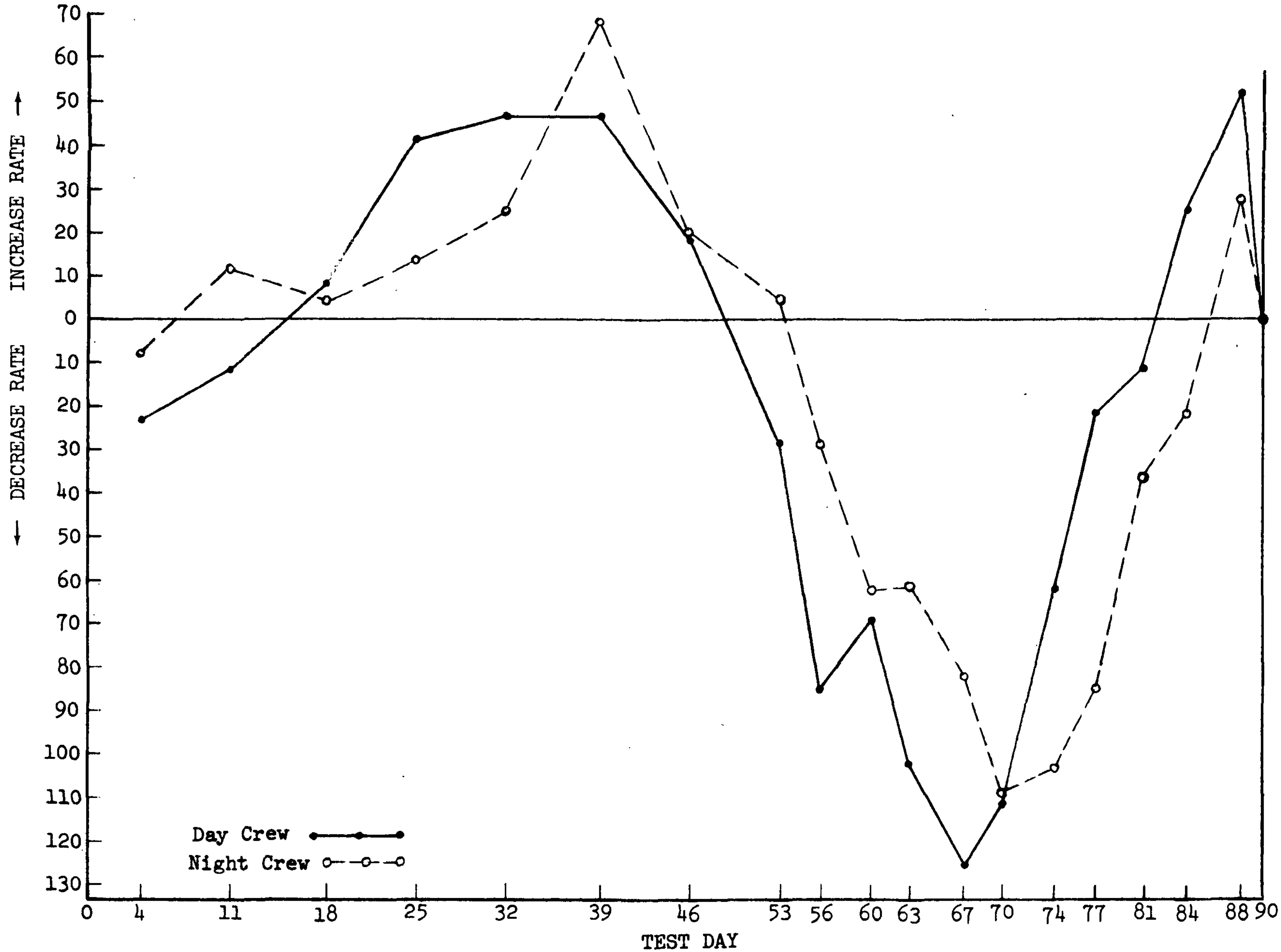

FIGURE 6. PLOT OF DIFFERENCE BETWEEN ACTUAL AND IDEAL ACCUMULATIVE EXCRETION RATE FOR 17-KGS

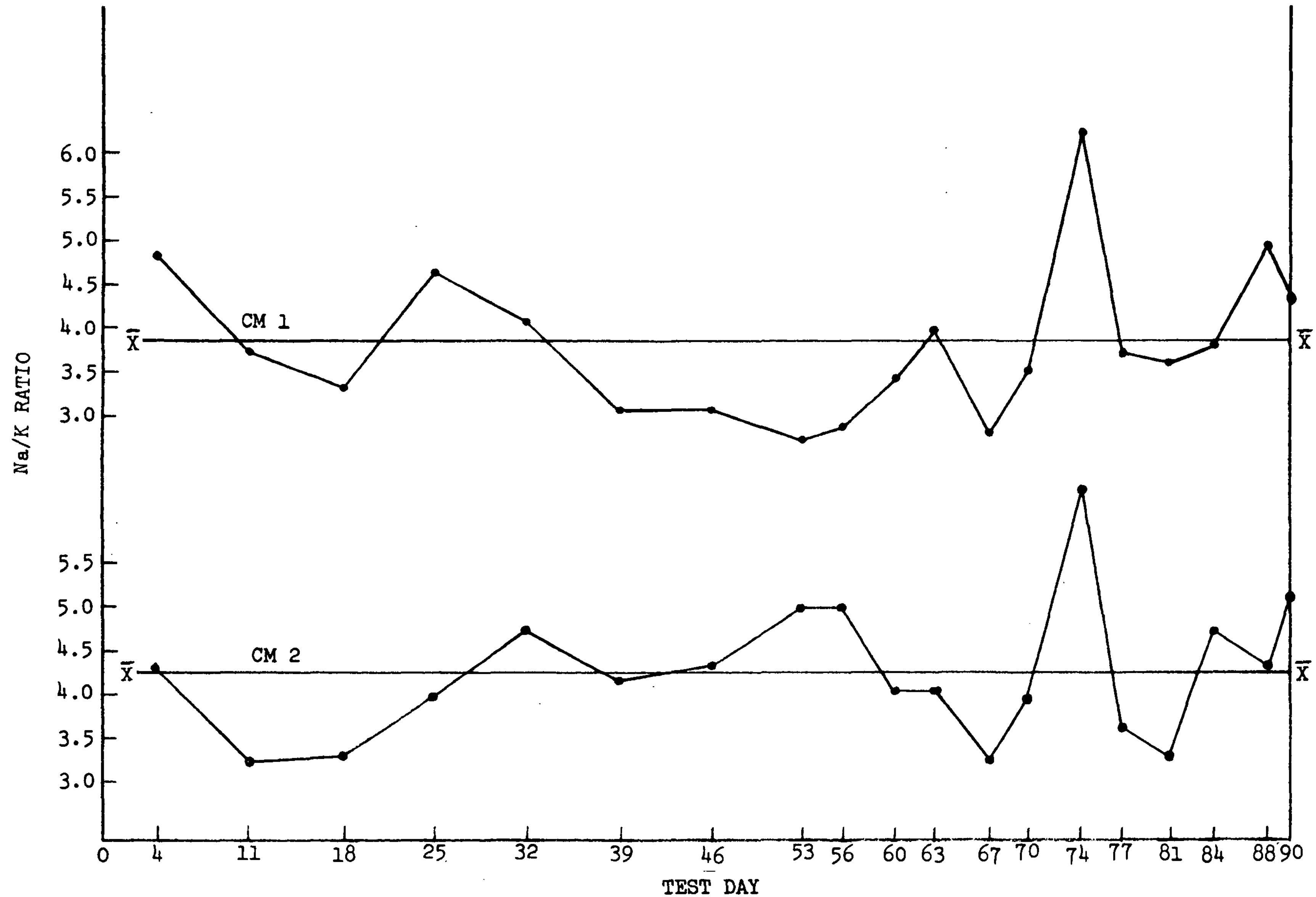

FIGURE 7. Na/K RATIO, CREWMEN 1 AND 2

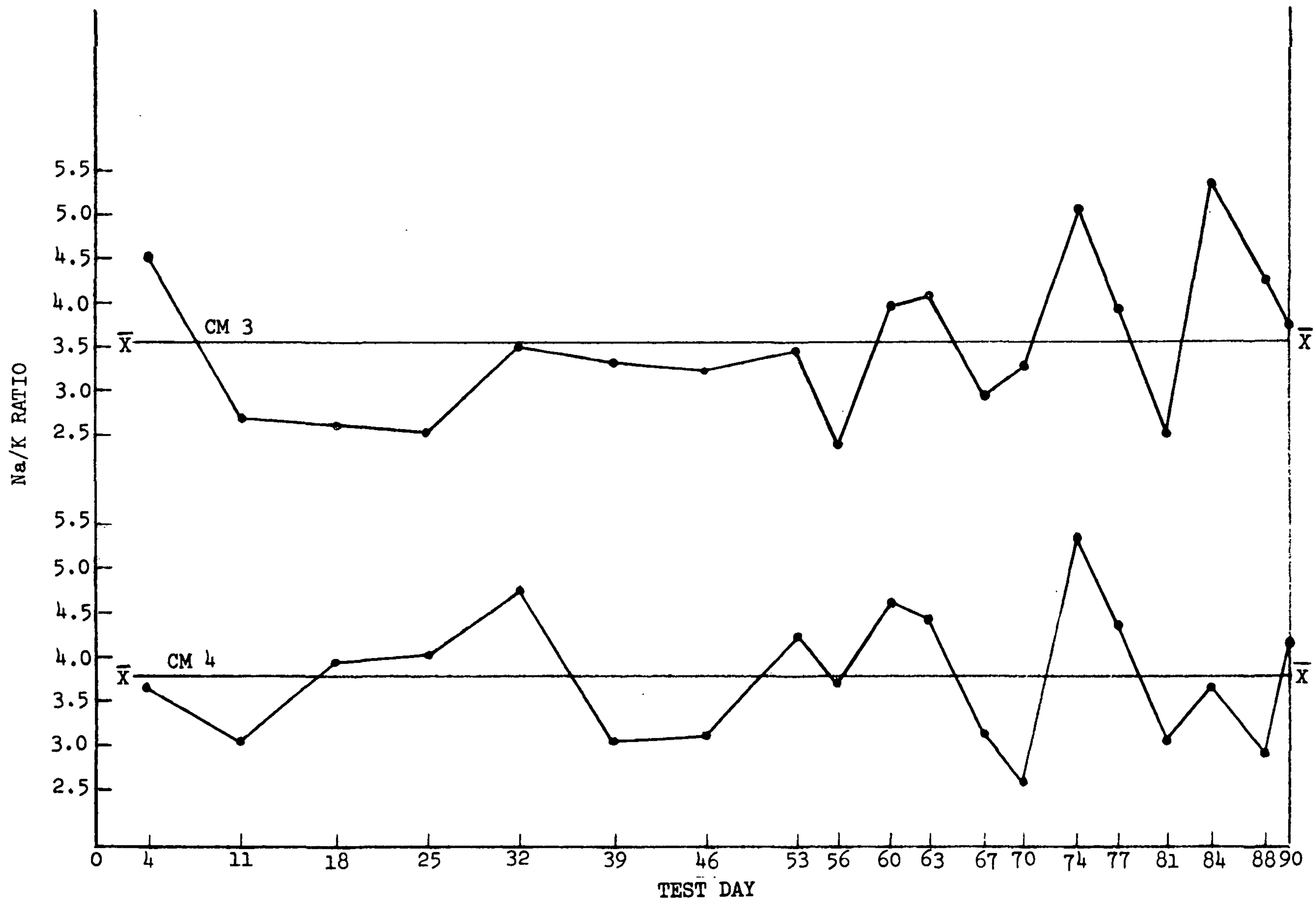

FIGURE 8. Na/K RATIO, CREWMEN 3 AND 4

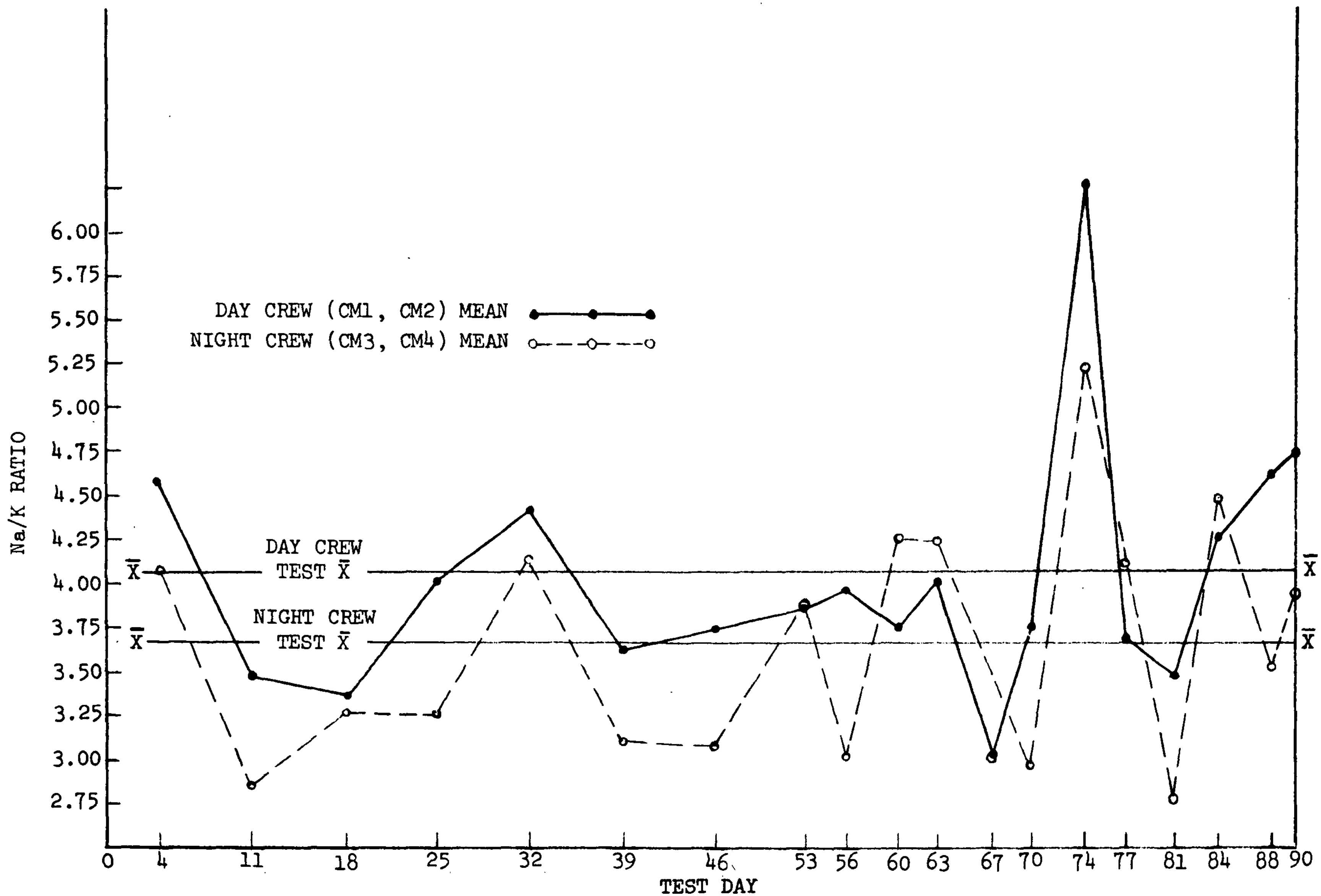

FIGURE 9. DAY VS. NIGHT CREW MEAN Na/K

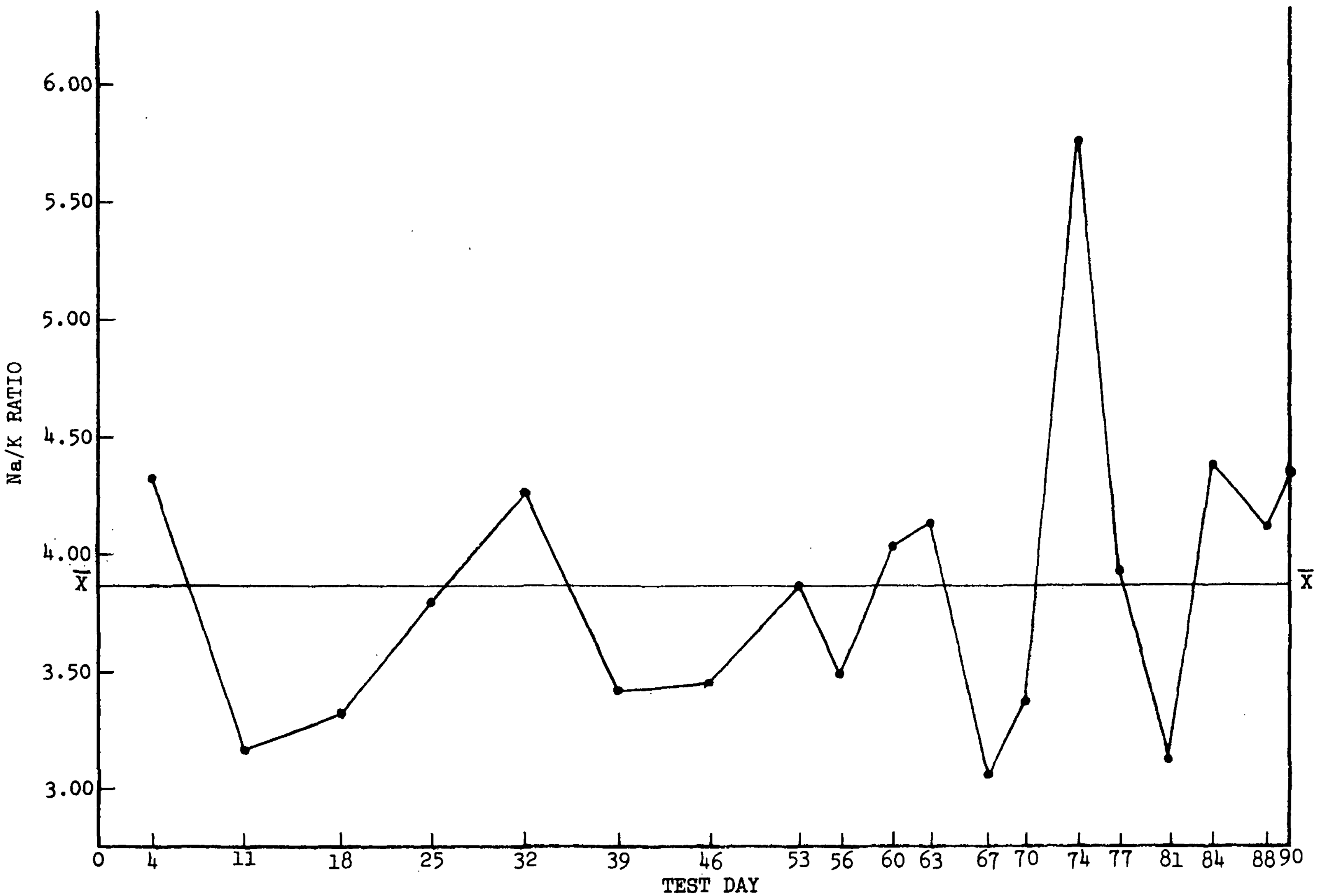

FIGURE 10. ALL CREW MEAN Na/K

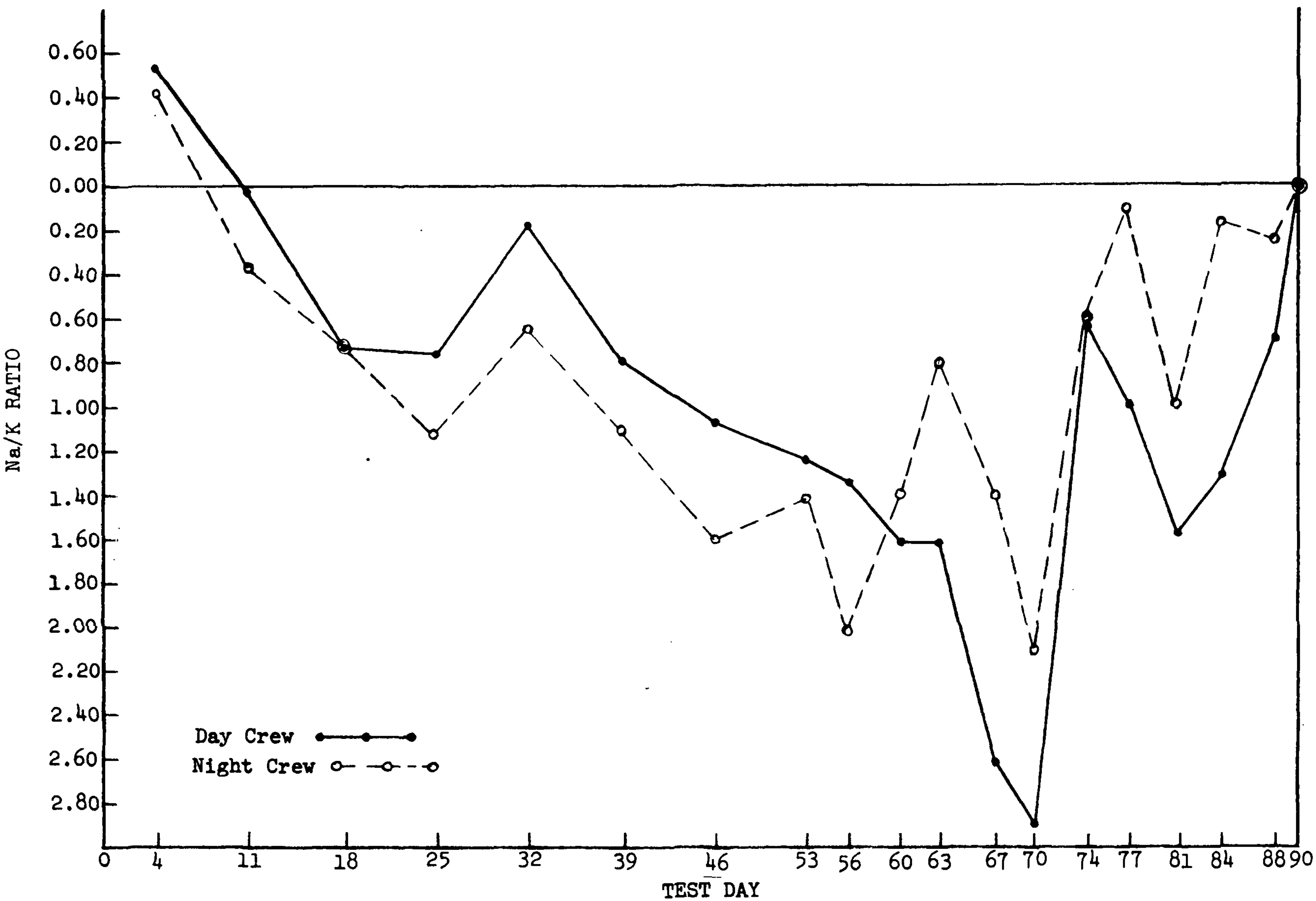

FIGURE 11. PLOT OF DIFFERENCE BETWEEN ACTUAL AND IDEAL ACCUMULATIVE Na/K

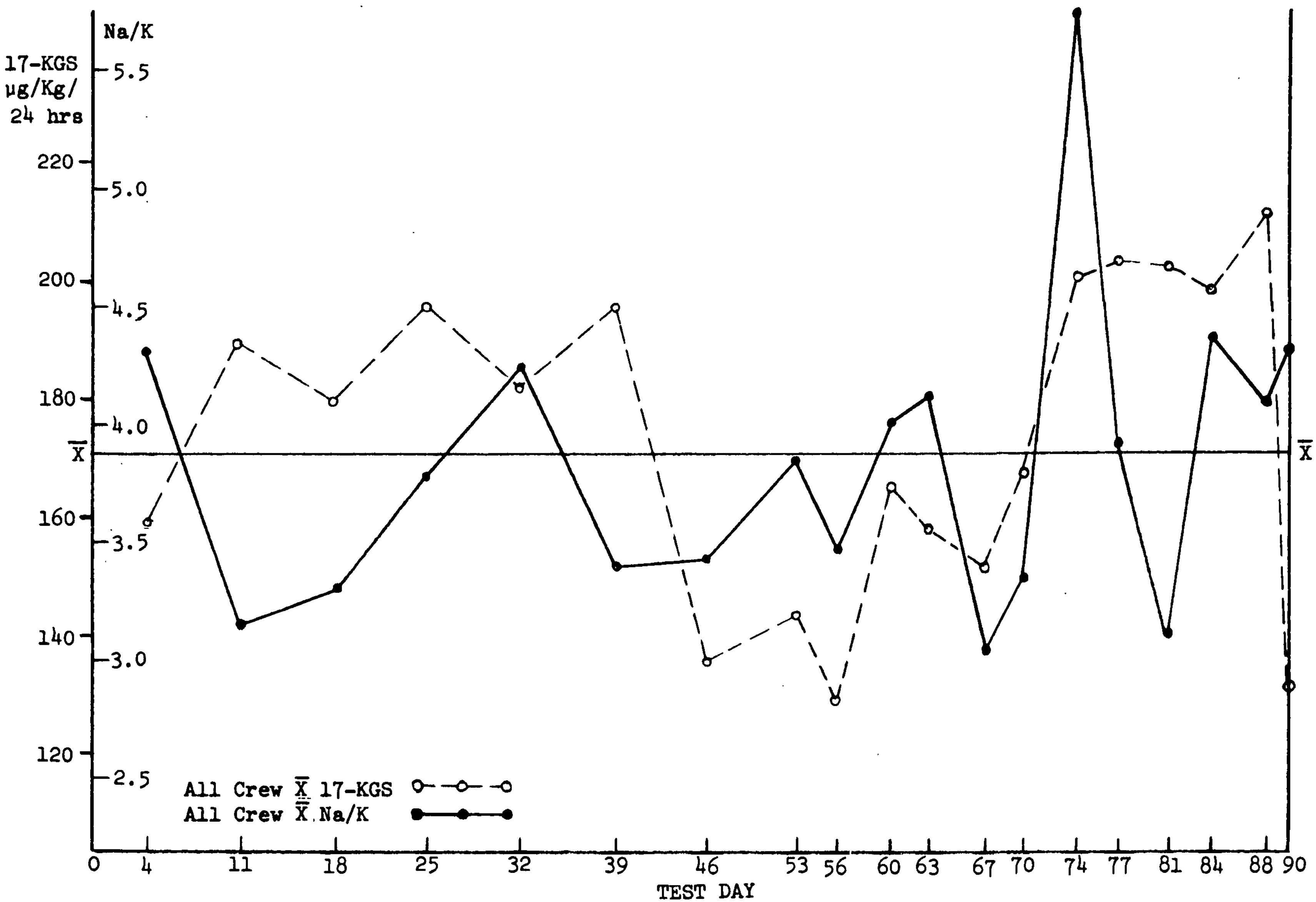

FIGURE 12. ALL CREW MEAN 17-KGS VS. Na/K

are presented in Figure 5. The same type of plot for each crew is shown in Figures 6 and 11 for 17-KGS and Na/K, respectively. These curves, being accumulative parameters, illustrate excretion rate changes. The all-crew rate plot for 17-KGS excretion, like the mean steroid concentration curve (Figure 4), shows discreet phasing over the course of the test. Figure 6 indicates rate curves of similar configuration for both crews demonstrating that they had approximately equivalent steroid excretion rates throughout the test. The day crew, however, appears to have been pacing the two groups in this presentation of the data since, except for the period between test day 18 and 32, they consistently reached the high and low points before the night crew. The accumulative difference plots for Na/K, unlike the steroid curves, do not show discreet phasing nor do they appear to have the same configuration for the two crews. After test day 46, the night crew curve (Figure 11) displays several short-term rate reversals not exhibited by the day crew and, unlike the steroid plot, neither crew appears to be pacing Na/K rate changes.

SECTION III

DISCUSSION

The test results indicate significant enhancement of adrenocortical acitivity of several weeks' duration at the beginning and at the end of the 90-day test. Although the lack of pre- and post-test data precludes discussion of the absolute significance of these findings, the crewmen were relatively hyperactive and presumably more stressed at these times as compared to the mid-test period. In view of the actual test operations, these results seem entirely reasonable. The first half of the test was marked by a continuing though declining workload, with considerable concern for completion of the test due to a succession of equipment malfunction problems. At about the time a decline in 17-KGS excretion occurs, however, it was apparent that completion of the 90-day test was a near certainty since key equipment had either irrevocably failed or was operating with a minimum of repair and maintenance. The elevation in steroid excretion during the last few weeks of the test is attributed to an anticipatory response (Reference 2) of impending test completion. The actual event is typified in the last sample as a release of tension by the abrupt fall-off in steroid excretion for all four crewmen. Steroid levels during actual flight circumstances (References 2 and 3) cannot be directly compared with our results since the referenced investigations utilized collection periods shorter than 24 hours. In general, however, lower 17-OHCS values have been associated with periods of reduced emotional and work loads while higher corticosteroid excretion has been correlated with periods of increased work level or with the psychogenic stress of exposure to a novel environment (References 3 and 4).

The Na/K data does not appear to have a direct relationship to the 17-KGS results except for the coincidental peak in the ratio at a time of rising steroid excretion during the period from days 67 to 74. This phase of the test has been characterized by the NIPA results (Reference 1) as a period of decline in crew morale somewhat ameliorated by the results of an "encounter session" undertaken at the instigation of the crew commander on day 69. Although the behavioral data support the contention that an improvement in morale began immediately after the discussion took place (which presumably carried the crew through to the end of the test), only the steroid data tends to corroborate such an immediate and sustained elevation in mood; the Na/K peak is quite short-lived and returns to the pre-encounter session level by day 81 (Figure 12). Compared to the Na/K data reported by Hale, et. al., (Reference 3), the all-crew mean ratio of 5.75 on day 74 is equivalent to the stress level attained by a C-135B aircrew during a 10-hour transpacific flight. Although Hales' stress spectrum for other groups of individuals under less stressful circumstances is consistent with our findings for only a few of the 90-day test samples, individual peaks in the ratio other than day 74 cannot be highly correlated with particularly stressful onboard events or, due to a lack of sensitivity, with the psychological findings. Again, comparing values for Na/K between this study and various operational flight situations, the results of Demos, et. al., (Reference 2) would appear to indicate that the 90-day crew spent a considerable portion of the test in a psychogenically stressed state equivalent to F-102 pilots anticipating a training or transoceanic flight; i.e., with Na/K ratios of 3.5 and higher.

SECTION IV

CONCLUSIONS

It may be concluded from the foregoing that 90 days of confinement, though not an extremely stressful situation, was not "a piece of cake" either, and "being on stage" for this extended period of time did have its emotional consequences. The results of this study indicate that long-term responses to non-specific stress may be followed satisfactorily by analysis of the urinary level of adrenocortical metabolites. In our experience, the Na/K ratio did not consistently correlate with steroid level nor did the mean ratio for the two groups of crewmen react in a similar fashion to the stresses of the test as was apparent for the steroid response. It is possible that the electrolyte ratio reflects short-term events not exhibited by the adrenocortical parameters or at least not of the same magnitude and timing. In this respect, the Na/K ratio would probably correlate better with an analysis of the sympathomedullary indices, adrenalin and noradrenalin (Reference 2).

SECTION V

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